MITOTIC RECOMBINATION IN THE HETEROCHROMATIN OF THE SEX CHROMOSOMES OF *DROSOPHILA MELANOGASTER*

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ABSTRACT

The frequency of spontaneous and X-ray-induced mitotic recombination involving the *Y* chromosome has been studied in individuals with a marked *Y* chromosome arm and different *XY* compound chromosomes. The genotypes used include *X* chromosomes with different amounts of *X* heterochromatin and either or both arms of the *Y* chromosome attached to either side of the centromere. Individuals with two *Y* chromosomes have also been studied. The results show that the bulk of mitotic recombination takes place between homologous regions.

MITOTIC recombination, as opposed to meiotic recombination, may take place in heterochromatic as well as euchromatic regions of the chromosomes and in males as well as in females (STERN 1936). Mitotic recombination was first detected in cells of the epidermal lineage, but was later also demonstrated to occur in the germ cells (FRIESEN 1936). In fact, many of the spontaneous translocations of chromosome fragments between the *Y* and *X* chromosomes and between their left and right arms possibly result from mitotic recombinational events (LINDSLEY 1955; see WILLIAMSON and PARKER 1976, for review). It has been thought that mitotic recombination results from breakage and rejoining of homologous chromosome regions. However, since recombination between nonhomologous regions would create aneuploid combinations with impaired viability, only those events generating quasi-euploid cells would be preferentially recovered. Thus, the assumption that mitotic recombination only occurs between homologous regions had to be revised. It has been suggested that the occurrence of mitotic recombination in Drosophila and other Diptera may be related to the existence of so called mitotic pairing between homologous chromosomes (STERN 1936). Although mitotic pairing can be directly observed only at prophase and metaphase, it is thought to reflect the pairing of homologous chromosomes during interphase, when mitotic recombination usually occurs.

In this paper we analyze the frequency of mitotic recombination between homologous regions as compared to that between nonhomologous ones. We have studied recombination between different heterochromatic regions of the *Y* chromosome, *in situ* in the *Y*, or appended to different arms of the *X* chromosome (*XY* compounds). Since unequal exchange between these regions generates viable

somatic cells, this analysis allows us to evaluate how homology affects mitotic recombination. The results indicate that the bulk of mitotic recombination occurs between homologous regions. Since the same arm can be attached to different places in the chromosome, we have studied how position affects frequency of mitotic recombination. The results indicate that position, and possibly pairing, determines recombination.

MATERIALS AND METHODS

We analyzed the frequency of appearance of mitotic recombination spots in *X/Y* males carrying in the *Y* chromosome the wild-type alleles of mutants present on either the *X* chromosome or an autosome. The cell-marker mutants used were yellow (*y, 1*; 0.0), which marks bristles, and multiple wing hairs (*mwh, 3*; 0.2), which marks trichomes.

The various chromosomes used in our study and their sources are listed below and are diagrammatically represented in Figure 1, which should enable those unfamiliar with Drosophila genetics to follow this work without concern about specialized terminology. Most of the chromosomes and mutants used are described in LINDSLEY and GRELL (1968). For our purposes, only the location of the three major heterochromatic segments of *X* and *Y* chromosomes is important. All inverted *X* chromosomes lack most of the centric heterochromatin except *Df(1)sc*^{8}, which carries most of the heterochromatic segment attached to the distal end of the left arm of the *X* chromosome (the position of the centromere is indicated by a raised dot). The two heterochromatic segments of the *Y* chromosome correspond to its long (Y^{L}) and short (Y^{S}) arms, defined here by the fertility factors they carry (*KL* and *KS*, respectively). They are attached either to the distal end of the euchromatin in the left arm of the *X* chromosome or to the small het-

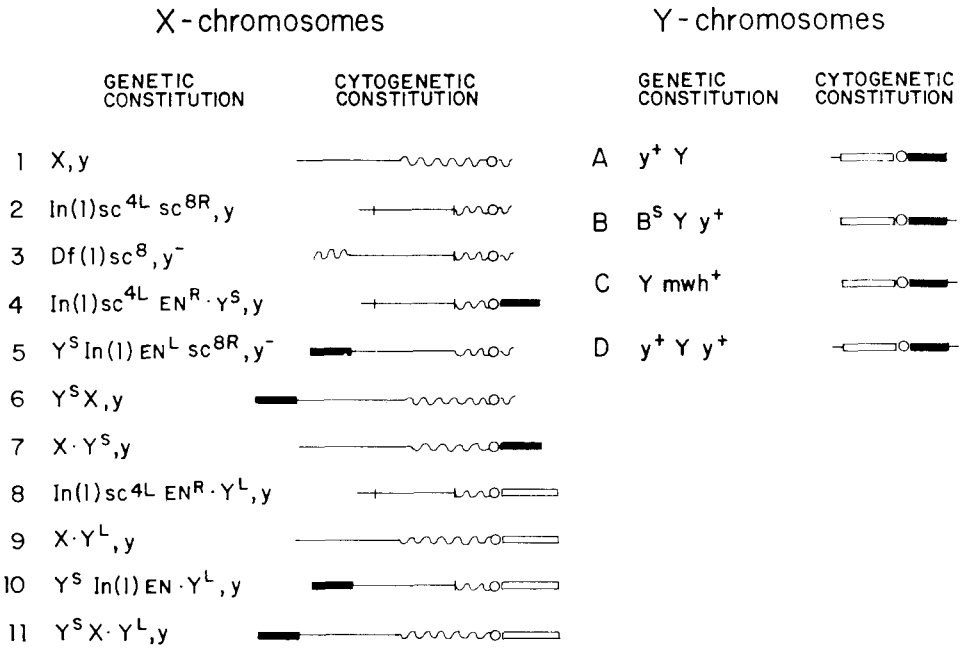

FIGURE 1.—Genetic and schematic cytogenetic constitution of the different sex chromosomes used. The numbers correspond to experiments as described in the text. Wavy line: *X* heterochromatin. Open bars: Y^{L}. Black bars: Y^{S}. Vertical lines represent inversion breakpoints.

erochromatic right arm. All free *Y* chromosomes used on this study have a small euchromatic duplication distal to the fertility factors of either or both arms. With the exception of B^S, all other duplications carry the wild-type allele of one of the cell marker mutants (yellow or multiple wing hairs).

X *chromosomes*

(1) *X·, y*, a normal sequence chromosome.
(2) *X·, In(1)sc^{4L}sc^{8R}, y bb$^-$* (Pasadena stock collection).
(3) *X·, Df(1)sc^8, y$^-$sc$^-$w^a* (Pasadena).
(4) *X·Y^S, In(1)sc^{4L}EN^R, cv y·KS*, recovered as a recombinant between *Y^{S}X·Y^L, In(1)EN, car f v cv y* (E. NOVITSKI) and *In(1)sc^4, y* (Pasadena).
(5) *Y^{S}X·, In(1)EN^{L}sc^{8R}, KS y$^-$ B w$^{a\cdot}$*, recovered as a recombinant between chromosome (3) and (10).
(6) *Y^{S}X·, KS y cv v f·* (Pasadena).
(7) *X·Y^S, y ac w^a ct^6 f·KS* (Chicago stock collection).
(8) *X·Y^L, In(1)sc^{4L}EN^R, y·KL* recombinant between chromosome (2) and (10).
(9) *X·Y^L, y w spl v f·KL*, recombinant between chromosome (1) and (11).
(10) *Y^{S}X·Y^L, In(1)EN, KS B y·KL* (Pasadena).
(11) *Y^{S}X·Y^L, KS y w v f·KL* (C. STERN).

All the $\overline{XY}$ chromosomes were kept in stock with *C(1)RM, y^2* and a marked (*y+* or B^S) *Y* chromosome. The presumed fertility factors present in the $\overline{XY}$ combinations were tested by crossing to $\overline{XX}$ females carrying either no *Y*, a Y^S fragment or a Y^L fragment ($Y^{L\cdot}sc^{S1}$, or *R(Y)L*; both from the Chicago stock collection).

Y *chromosomes*

(A) *y+Y, l(1)J1+ — ac+ KL·KS* (Pasadena).
(B) *B^{S}Y y+, B^S su(f)+KL·KS ac+ — l(1)J1+* (Pasadena).
(C) *Y mwh+, Dp(3;Y) P6, KL·KS mwh+* (LEWIS 1969; RIPOLL and GARCIA-BELLIDO 1973).
(D) *y+ Y y+, y+ KL·KS y+* (D. PARKER).

The $\overline{XY}/Y$ males were generated in crosses of $\overline{XY}/Y$ males (1–11) to $\overline{XX}$, *C(1)RM, y^2* females carrying a *Y* chromosome of type *y+Y* (A series) or type *B^{S}Y y+* (B series). In the C series, *C(1)RM, y^2/Y mwh+*; *mwh ve h* females were crossed to *XY* males homozygous for *mwh jv*. Since both parental stocks carried marked *Y* chromosomes, exceptional F_1 males

TABLE 1

Frequency of yellow or multiple wing hairs spots per one hundred abdomens

	Genetic constitution	*X*	*Y*	Series A *y+ Y* *n*	*y*	*mwh*	V_s	V_i	Series B *B^{S}Y y+* *n*	*y*	*mwh*	V_s	V_i	Series C *Y mwh+* *n*	*mwh*
1	*X·, y*			131	5	6	8	8	162	7	1	11	8	208	8
2	*X·, In(1)sc^{4L}sc^{8R}, y*			153	8	2	60	50	83	15	7	44	32		
3	*X·, Df(1)sc^8, y$^-$*			44	27	9	100	100	62	30	6	98	100	69	13
4	*X·Y^S, In(1)sc^{4L}EN^R, y*			94	4	3	25	46	40	17	5	28	—	48	12
5	*Y^{S}X·, In(1)EN^{L}sc^{8R}, y$^-$*			49	22	8	47	47	87	32	6	80	73		
6	*Y^{S}X·, y*			104	21	4	30	30	47	34	4	19	37		
7	*X·Y^S, y*			42	7	2	6	20	49	4	6	20	—		
8	*X·Y^L, In(1)sc^{4L}EN^R, y*			48	33	4	12	17	48	16	2	4	20	72	18
9	*X·Y^L, y*			57	19	7	10	18	47	11	8	4	18		
10	*Y^{S}X·Y^L, In(1)EN, y*			63	66	8	6	5	30	143	13	2	10	53	143
11	*Y^{S}X·Y^L, y*			68	22	9	10	10	59	40	8	3	6		
12	*In(1)sc^{4L}sc^{8R}, y/Y mwh+*			74	31	31	5	—	46	22	20	6	—		

n: number of abdomens. V_s and V_i, frequency of *y* spots (possibly due to variegation) in the genitalia (claspers) of the same abdomens; V_s: spontaneous, V_i: irradiation experiments.

($\overline{XY}/Y/Y$) could be recognized and they were discarded. The males of experiments 1 to 11 were generated by these crosses (Table 1). In experiment 12, $X/Y/Y$ males genetically $In(1)sc^{4L}sc^{8R}/B^{s}Yy+/Y$ *mwh*+; *mwh ve h/mwh jv* (A series) or $In(1)sc^{4L}sc^{8R}/y+Y/Y$ *mwh*+; *mwh ve h/mwh jv* (B series) were generated by crossing $X, y/Y$ *mwh*+; *mwh ve h* males to $In(1)sc^{4L}sc^{8R}$ females with either the $B^{s}Y\ y+$ or the $y+Y$ chromosome and homozygous for *mwh jv*.

All the X chromosomes studied are viable in XO males with the following exceptions: chromosomes (2) and (8) are entirely or partially deficient for bobbed. Chromosomes (3) and (5) are deficient for the loci located distal to the left breakpoint of $In(1)sc^{s}$. This deficiency is viable in cells (GARCIA-BELLIDO and SANTAMARIA 1978) in deficiency//normal mosaic individuals.

Irradiation of males in the different experiments was carried out with a Phillips MG/15 Be (150 KV, 15 mA, 2 mm Al filter) at a rate of 320 r/min. Since the imaginal cells of the tergite do not divide during larval development (GARCIA-BELLIDO and MERRIAM 1971), the data from males irradiated as larvae of different ages were pooled. Adult males were mounted in euparal and examined under the compound microscope. Only tergites II-VI were scored, and only spots including two or more bristles were recorded in order to avoid that fraction of the clones with impaired viability, or that fraction due to phenocopies or recombinational events in the pupa (GARCIA-BELLIDO 1972).

RESULTS

The purpose of this work was to determine the relative frequency of mitotic recombination between different regions of the Y chromosome. The appearance of either y or *mwh* marked spots in XY/Y males can result from mitotic recombination between (1) identical regions of the Y chromosome, one region in the Y arm carrying the marker and the other translocated to the X chromosome, or (2) from nonspecific recombination between the marked arm of the Y chromosome and any other heterochromatic or euchromatic region in the genome: (a) If recombination with the Y chromosome takes place within the proximal heterochromatin of the X chromosome, the marked recombinant cells are expected to be female. (b) Recombinant cells will be aneuploid for different regions of the X chromosome if recombination with the Y chromosome takes place in the euchromatic region of the X chromosome. (c) Recombination of Y chromosome with the autosomes will generate marked euploid or aneuploid cells. Another cause of marked spots will be nonreciprocal loss of a marker following: (d) Y-chromosome sister-chromatid exchanges, (e) fusion between Y^{L} and Y^{S} arms, (f) Y-chromosome sister-chromatid fusion on the same arm, or (g) Y-chromosome loss. (3) Finally, variegation for the markers could spuriously generate marked clones of cells that could be considered a consequence of mitotic recombination events.

The following experiments allow us to evaluate the fraction of events that generate marked spots that result from mitotic exchanges between identical regions of Y chromosome (1) and those caused by any of the other mechanisms described above (2 and 3). Variegation (3) was monitored by studying y spots in the genitalia (claspers) and *mwh* spots in the wing. As we indicate below, y spots in the genitalia are almost exclusively due to variegation. The occurrence of marker loss by mechanisms 2c and 2g was estimated by studying the occurrence of y spots in $y/y^{+}\ Y\ y^{+}$; *mwh*/+ males. No spontaneous y spots were found in 26 male abdomens although four y spots were found in 57 abdomens after exposure to 1000r of X rays. In the same males, the number of *mwh* (control)

spots were 1 and 46, respectively. This indicates that mechanisms such as chromosome loss and sister-chromatid fusions account for a very small fraction of the marked spots (compare with Table 1 and Figure 3).

(1.) *Spots of spontaneous origin*

In order to investigate the amount of nonspecific recombination between the *Y* chromosome and the other chromosomes, we have analyzed the effects of variation in the amount and position of the heterochromatin of the *X* chromosome, leaving the rest of the genome unchanged. Thus, a particular *Y*-chromosome fragment was studied in genotypes carrying different *X* chromosomes (see Figure 1).

Table 1 and Figure 2 show the frequencies of *y* and *mwh* spots found in males of different genotypes. In series A and B, *mwh* spots correspond to mitotic recombination events in the left arm of chromosome 3. The frequency of such spots is, therefore, used as an internal control. As seen in Table 1 and Figure 2 these frequencies do not vary significantly. Table 1 shows the percent of male genitalia with *y* spots in the different experiments. These frequencies vary markedly between genotypes. These spots could result from mitotic recombination events or from variegation of the y^+ gene translocated to the *Y* chromosome. That these spots correspond to variegation is supported by two facts: (1) in general their frequency does not vary with X-irradiation (Table 1) and (2) the variations in frequency are inversely correlated with the amount of heterochromatin present

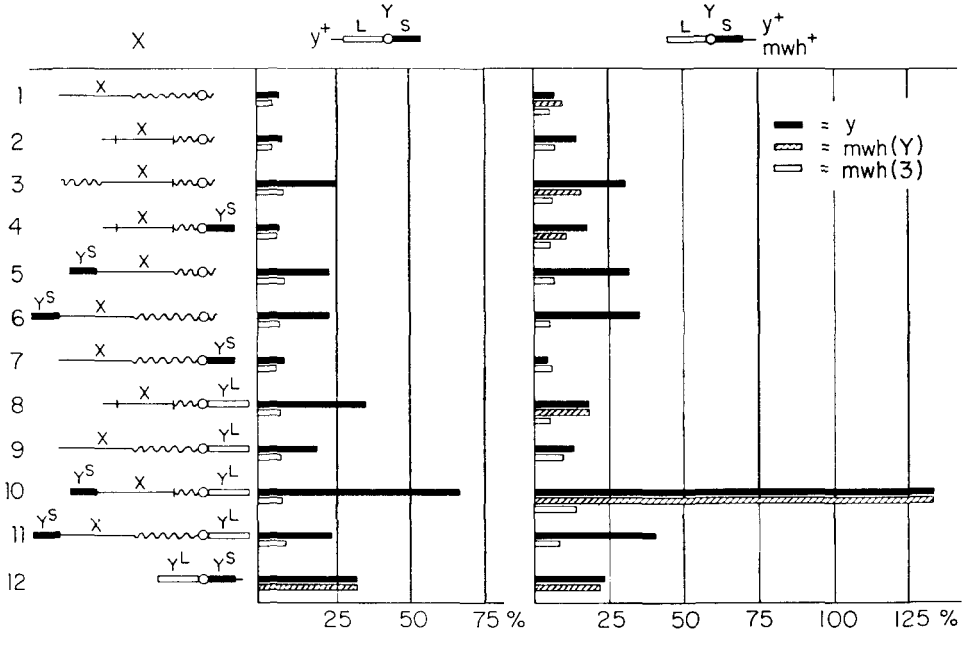

FIGURE 2.—Frequency of spontaneous spots found in different genetic combinations. Frequency as number of clones per one hundred abdomens. Schematic representation of the chromosomes as in Figure 1.

in the genome. In this respect, it is the absence of X heterochromatin near the centromere that seems to correlate with the highest frequencies of spots (experiments 2, 3, 4 and 5). X heterochromatin that is distally attached seems to increase variegation (experiments 2 and 3). Similarly, variegation increases when a Y^S fragment is distally attached to a normal X chromosome (compare experiments 1, 5 and 6). In experiment 3, when the y^+ marker is in either the Y^L (Series A) or Y^S (Series B) arm the frequency of variegation is very high. It is surprising that in *In(1)sc^s/Y* males variegation for yellow is lower (54%) than in the other combinations, although all three are genetically similar. The presence of a Y^L fragment attached to the X chromosome suppresses variegation more effectively than the presence of a Y^S (compare experiments 8 with 4 and 5). The duplicated element of *mwh*$^+$ in the Y^S (*Y mwh*$^+$) does not seem to be exposed to variegation. This was tested in *mwh*$^+$ Y^SX·/0; *mwh* males (GARCIA-BELLIDO and RIPOLL 1973) and in *In(1)sc^{4L}sc^{8R}/sc^{V1}Ymwh*$^+$; *mwh* males (RIPOLL and GARCIA-BELLIDO 1973).

We assume that variegation also takes place in the tergites. If this variegation correlates in both tissues, a fraction of the spots in the tergites can be considered to be due to variegation. Since the *mwh*$^+$ duplication in the Y chromosome does not variegate, we can evaluate in some experiments the fraction of yellow spots due to variegation (*cf.*, expts. 3B and 3C, on the one hand, and 8B and 8C on the other). Apparently the fraction of yellow spots in the abdomen due to variegation is negligible in genetic combinations showing, in the genitalia, a variegation frequency less than 30%.

The different genetic combinations studied can be subdivided into groups: X chromosomes without Y fragments (1 to 3), X chromosomes with Y^S fragments (4 to 7), X chromosomes with Y^L fragments (8 and 9) and X chromosomes with both Y^S and Y^L fragments (10 and 11). The data in Table 1 and Figure 2 uncover several general rules. For the different X chromosomes without Y fragments (experiments 1, 2 and 3), the frequencies of spots are similar in series A (Y^L), B and C (Y^S). These results suggest that neither the Y^L nor the Y^S arms specifically recognize the X chromosome. If there is any specific distinction between different X chromosome configurations, they are blurred by nonspecific recombinations with the rest of the genome. Mitotic recombination between X chromosomes carrying Y^S fragments and the Y^S arm is higher than that with a Y^L arm in experiment 2. However, in an analogous combination (experiment 7) this relationship is not noticeable. A similar behavior to that of experiment 4 is observed between Y^L fragments and the Y^L arm (experiments 8 and 9). In experiments 10 and 11 the presence of both Y^S and Y^L fragments increases recombination with either Y chromosome arm. These results are consistent with the notion that the different Y arms specifically recognize the presence of the same Y arms attached to the X chromosome.

The hypothesis of specific chromosome arm recognition was tested in experiment 12. In this experiment, we can evaluate mitotic recombination between identical arms because of the generation of y and *mwh* twin spots. In series B, *mwh* and y spots are expected to appear as twin spots because both markers are

in the same chromosome arm, whereas twin spots in experiment A are indicative of nonspecific recombination. In experiment B, twins represent 66% (6/9) of all the *mwh* spots, and in experiment A twin spots represent only 13% (3/23) of all the *mwh* spots. These results reinforce the idea that mitotic recombination in the Y chromosome preferentially takes place between identical arms.

The position of the Y fragments in the X chromosome seems to influence the frequency of recombination with the arms of the Y chromosome. In series A and B (and C) the spot frequency is equal in chromosomes with and without a Y^S fragment translocated to the right arm of the X (compare experiment 2 with 4, and 1 with 7). However, when the Y^S is translocated to the tip of the left arm of the X chromosome, the frequency of mitotic recombination with the Y^S is substantially increased (experiments 1 and 6; 2 and 5). The presence of Y^L in the right arm of the X leads to an increase in the frequency of recombination with the Y^L arm (experiments 1 and 9; 2 and 8).

The very high frequency of recombination found in experiment 10 compared with the rest is surprising, especially when compared with experiment 11 which differs only in the amount of proximal X-heterochromatin. This higher fre-

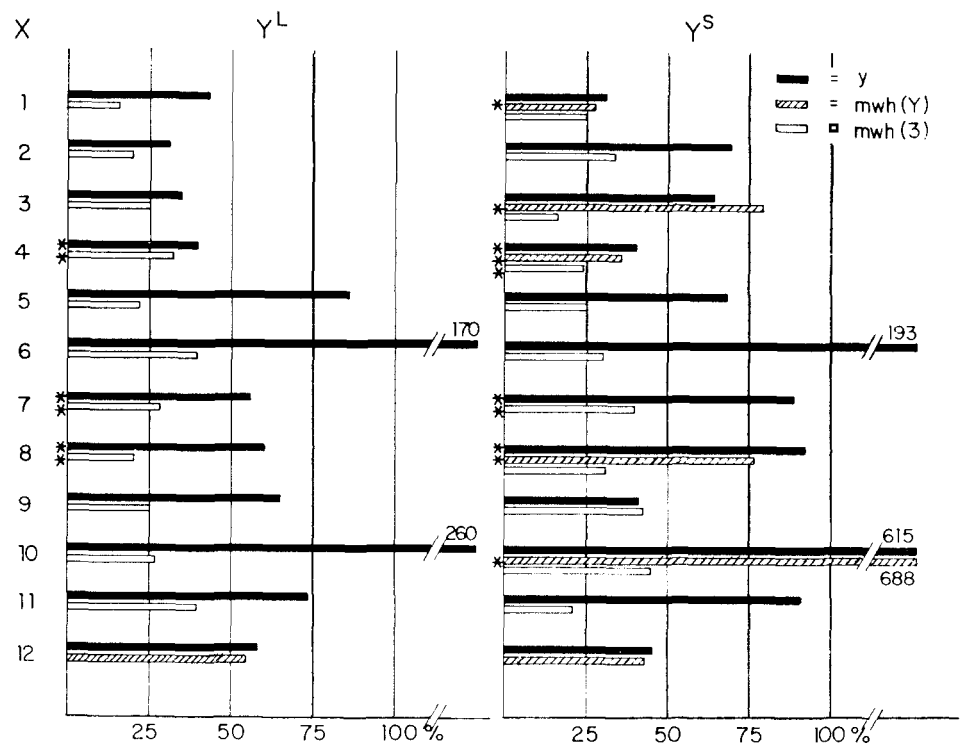

FIGURE 3.—Frequency of spots found in different genetic combinations after 500r X irradiation. Frequency as number of clones per one hundred abdomens. The numbers (1 to 12) correspond to experiments as described in Figures 1 and 2.

*, irradiated with 1000r and corrected for 500r.

quency is not due to a general increase in mitotic recombination, because the frequency of *mwh* spots arising from recombination in the *3L* chromosome arm is comparable to that found in other experiments. In addition, chromosomes (5) and (8), which are derivatives of chromosome (10), do not show high frequencies of recombination.

(2). *Spots after X irradiation*

The same genotypes presented in Table 1 were studied in flies following X irradiation. Most of the experiments were done using a dose of 500r, some with 1000r (Figure 3). The data obtained from experiments where a dose of 1000r was used have been corrected to that expected for 500r, assuming a two-fold difference between 500 and 1000r. Following irradiation, the fraction of y spots due to variegation becomes negligible (experiments 3 and 4). Most of the generalizations that were found when studying nonirradiated flies are still valid after irradiation, although differences between Y^L and Y^S are somewhat blurred, probably due to an increase in nonspecific recombination. However, arm specificity is still maintained. In experiment 12 B, twin spots represent 42% (5/12) of all the *mwh* spots, and in experiment 12 A, where twin spots reflect anisobrachial recombination, only 11% (2/18) of the *mwh* spots are found as twins with a y spot.

DISCUSSION

Two cell markers have been used to monitor the occurrence of mitotic recombination in the *Y* chromosome: One is y^+, the other $mwh^+ \cdot y^+$ is located in a translocation of the tip of $In(1)sc^s$ to the *Y* chromosome. The translocated element probably carries part of the centric *X* heterochromatin to either the long (y^+ *Y*) or the short (B^S *Y* y^+) arm of the *Y* chromosome. In both cases, y^+ is distal to the *Y* chromosome fertility factors. mwh^+ is in a duplication, one of the elements of the *T(Y;3)P6*, which is a reciprocal translocation between the tip of *3L* and the short arm of the *Y* chromosome. This duplication is also distal to the fertility factors of Y^S (RIPOLL and GARCIA-BELLIDO 1973). But whereas mwh^+ does not variegate, y^+ does. It is not surprising that, since y^+ variegates in $In(1)sc^s/Y$ males, it will also variegate when translocated to the *Y*. It was, however, surprising that this variegation increased with variations in the constitution of the *X* chromosome. Not only did the y^+ in the *Y* chromosome variegate in the presence of $In(1)sc^{4L}sc^{s^R}$, which lacks most of the centric heterochromatin, but it also variegated even more with $Df(1)sc^s$, which retains most of the *X* heterochromatin (PANSHIN 1938; *vid* SPOFFORD 1976). The role of different *Y* regions in their effects upon suppression or enhancement of variegation was analyzed by BAKER and SPOFFORD (1959).

Except for those cases where yellow spots result from variegation, the loss of the y^+ or mwh^+ marker in the *Y* chromosome should result from either somatic mutation (a presumably rare event) or from chromosomal recombination events. These are expected to be of two main classes, either chromosome loss or recombination between the arm carrying the marker and any other chromosome region,

followed by segregation and the formation of aneuploid cells. Aneuploidy involving only regions of the *Y* chromosome is expected to be compatible with cell viability, whereas aneuploid conditions involving *X* chromosome or autosome regions are potentially lethal. Recombination between nonidentical chromatids will create, depending on the polarity of the exchange, either dicentric bridges (interchange with opposite polarity) or reciprocal aneuploids (interchange with matching polarity). In the latter case twin elements, either hyperploid or hypoploid for the region distal to the interchange, will be generated. The effect on cell viability of dicentric bridges is not known, but is expected to lead to hypoploid cells by loss of both centromeres or to centric fragments with variable amounts of genetic material in each break-reunion cycle. When recombination interchanges terminal regions, the viability of the aneuploids depends on their genetic natures (hypoploids or hyperploids).

In our experiments, we could not genetically distinguish recombinational events involving the *Y* chromosome from those between the *Y* and either the *X* chromosome or an autosome. However, the fraction of either yellow or multiple wing hairs spots increases several times when there are present in the genome, together with the marked *Y*, other *Y* chromosomes or *Y*-chromosome fragments attached to the *X* chromosome. This finding suggests that most recoverable spots are due to exchanges between identical chromosome regions. That this is not spuriously due to the lethality of the other recombinant types mentioned above is supported by the following arguments. *Y*-chromosome loss must be a rare event, for even following treatment with 1000r of X-rays, the frequency of yellow spots in $y^+ Y y^+/X, y$ males is extremely low. The same experiments indicate that exchanges with opposite polarity must be rare events; interchange between the two arms of the *Y* chromosome in $y^+ Y y^+$ will go unnoticed if it occurs between sister chromatids or between the same chromatid if the interchange retains the chromatid polarity, but ring *Y* chromosomes with loss of both y^+ markers would occur if that interchange is with opposite polarity.

Interchange between the *Y* and *X* chromosome or any autosome will generate aneuploid cells. However, in all cases those marked with yellow correspond to hyperploids. Hyperploidy for large autosomal regions does not have any effect upon cell viability (RIPOLL, unpublished) and even individuals hyperploid for two-thirds of an autosome may occasionally survive (LINDSLEY *et al.* 1972). Males hyperploid for small regions of the *X* chromosome can be viable (PATTERSON, STONE and BEDICHEK 1937), and male cells hyperploid for large regions of the *X* chromosome have been reported to be viable in sex mosaics (PATTERSON and STONE 1938). Thus, the majority of the hyperploid cells that can be generated are expected to be detectable. Therefore, when compared with the frequency of marked spots arising from somatic exchanges between *Y* chromosomes, the fraction of spots due to nonhomologous exchanges between the *Y* chromosome and either euchromatic or heterochromatic regions of other chromosomes must be very low. As we have seen above, this is true even after irradiation.

These results are comparable to others carried out in females. WALEN (1964) studied the frequency of yellow and singed (*sn*) twin spots following spontaneous interchange between different combinations of *X* chromosomes carrying different amounts of *X* heterochromatin and different arms of the *Y* chromosome appended to the *X* chromosome. In fact, she used our chromosomes (2), (8), (10) and (11) and a ring chromosome. However, since the markers used, *y* and *sn*, were proximal to or in the opposite arm to that of the appended *Y* arms, she could conclude only that the presence of heterochromatin of either the *X* or the *Y* chromosomes increased the frequency of interchanges. For the same reason, specificity of exchange between the three different heterochromatic segments (those of the *X*, Y^S and Y^L arms) could not be ascertained. Similar uncertainties regarding specificity of exchange resulted after irradiation of females with different *XY* combinations and using white as a cell marker (JANNING 1970).

The experiments reported in this paper allow us to determine possible specificities for exchanges between arms of the *Y* chromosome and the centric heterochromatin of the *X* chromosome. We have compared frequencies of spots in males carrying in the *Y* chromosome one marked arm (either with y^+ or mwh^+) and in the *X* chromosome either Y^S, Y^L, or both, appended distally. The frequency of yellow or multiple wing hairs marked spots due to interchanges (*i.e.*, corrected for variegation) between the *Y*-chromosome arms or between either arm of the *Y* chromosome and the *X* chromosome and autosomes is markedly different (Figure 2). This frequency is much lower in males in which there is no *Y* chromosome arm appended to the *X* chromosome than in those in which one arm, the other, or both, are present.

The frequency of yellow or multiple wing hairs spots in males without *Y*-chromosome arms attached to the *X* chromosome is equally low when the centric *X* heterochromatin is lacking as when it is attached distally (and in reverse sequence). That low frequency of spots includes those produced by interchanges of a *Y*-chromosome arm, not only with the *X* chromosome (heterochromatin and euchromatin) and with the autosomes, but also with the opposite arm of the *Y* chromosome. Since the presence of either *Y* arm attached to the *X* chromosome increases the frequency of spots produced by exchange between that *Y* arm and either marked *Y*-chromosome arm, we conclude that the majority of the spots in males without extra *Y* fragments results from anisobrachial interchange between the two arms of the *Y* chromosome. If this is correct, pairing between the *X* heterochromatin and the heterochromatin of either arm of the *Y* chromosome must be low, and on the contrary both *Y*-chromosome arms must pair nonspecifically to some extent. Based on the frequency of exchanges in premeiotic cells, LINDSLEY (1955) reported a higher degree of homology of the *X* heterochromatin with the short arm than with the long arm of the *Y* chromosome. We have not been able to detect such a difference. It should be kept in mind, however, that exchanges in the proximal heterochromatin of the *X* chromosome will yield female cells, and the possibility exists that these cells are not viable in somatic spots due to unbalanced dosage compensation. Other experiments have shown

that exchange between identical arms is more frequent than that between opposite arms. The fraction of Y-arm specific exchanges was directly measured in males with two marked Y chromosomes and an $In(1)sc^{4L}sc^{8R}$ X chromosome to avoid possible interference with the X heterochromatin (experiment 12A and B). The analysis of twin spots suggests that a majority of the spots result from exchanges between identical arms.

Homology is, however, not the only requisite for exchange, as shown by comparing experiments 6 and 7. A Y^S arm terminally attached to either the left or the right X-chromosome arm shows very different frequencies of exchange with the free Y chromosome. Thus, the existence of pairing specificity of heterochromatin for the occurrence of mitotic recombination suggests that identity (possibly DNA sequence or chromatin architecture) is a requisite for pairing and pairing in turn is required for recombination.

Related to this conclusion is the question of whether the amount of recombination is proportional to the length of the identical regions involved. Y^L exchanges are apparently as frequent as Y^S exchanges for comparable XY combinations. The frequency of interchanges between identical regions (substracting from experiment 8A or 9A the frequency of 1A, and from experiments 5B and 6B, that of 1B) is about 25 spots per 100 abdomens. It is interesting to notice that this is the frequency of spontaneous recombination in the centric heterochromatin in females (24% of forked spots, GARCIA-BELLIDO 1972). These considerations suggest that (1) the centric heterochromatin of the X chromosome shows pairing specificity, and (2) the frequency of recombination is roughly proportional to the length of the paired segment.

X irradiation does not alter these general properties of mitotic pairing and exchange. A quantitative analysis of the data presented in Figure 3 shows that Y-arm specificities become somehow obliterated. It is possible that X irradiation gives rise to a larger fraction of interchanges between nonhomologous sequences. In fact, data from irradiation experiments in the germ line, where Y chromosome fragments can be followed to either the X chromosome or the autosomes, seem to agree with that notion (see review by WILLIAMSON and PARKER 1976).

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